American Journal of Medical Genetics (Neuropsychiatric Genetics) 67:202–207 (1996)

Maternal Inheritance and Chromosome 18 Allele Sharing in Unilineal Bipolar Illness Pedigrees

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We have replicated the observation of McMahon et al. [1995] that there is excess maternal transmission of illness in a series of previously described unilineal Bipolar manic-depressive illness extended pedigrees [Berrettini et al., 1991]. ("Transmission" is defined for any ill person in a pedigree when father *or* mother has a personal or immediate family history of major affective disorder.) We divided our pedigrees into exclusively maternal transmission (Mat) and mixed maternal-paternal transmission (in different pedigree branches) (Pat). Using affected sib-pair-analysis, linkage to a series of markers on chromosome 18p-cen was observed in the Pat but not the Mat pedigrees, with significantly greater identity by descent (IBD) at these markers in the Pat pedigrees. As compared with the pedigree series as a whole, the proportion of alleles IBD in the linkage region is much increased in the Pat pedigrees. As shown by Kruglyak and Lander [1995], as the sharing proportion of alleles in affected relative pairs increases, the number of such pairs needed to resolve the linkage region to a 1 cM interval becomes smaller. Genetic subdivision of an illness by clinical or pedigree configuration criteria may thus play an important role in discovery of disease susceptibility mutations

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KEY WORDS: genetic linkage, affected-sib-pairs, manic-depressive illness

INTRODUCTION

McMahon et al. [1995] recently proposed the novel hypothesis that there is excess maternal transmission in bipolar illness, in families selected at Johns Hopkins University for apparent unilineal transmission, and that this was consistent with gene imprinting or mitochondrial inheritance. We have a series of bipolar pedigrees selected for unilineality in a similar manner [Berrettini et al., 1991] and have attempted to replicate the observations of McMahon et al. [1995]. By unilineal, we refer to pedigrees where in the most ancestral couple not more than one person is ill, and no person marrying into the pedigree, or the marrying-in person's first-degree relatives (other than children) has a diagnosis within a broadly defined affective spectrum. To determine "transmission," pedigrees are inspected to determine whether a male or a female is the likely transmitting parent (see Method description below).

Our Bipolar pedigree series is also similar to the series studied by McMahon et al. [1995] in that linkage to markers on chromosome 18, first reported by Berrettini et al. [1994] has been replicated in the Hopkins series, with similar significance levels at certain markers [D18S40, D18S37; Stine et al., 1995].

The Hopkins pedigree series consists of less extended pedigrees than our own, so that heterogeneity of observed transmissions within a pedigree would occur more easily in our own series (that is, in one branch of the pedigree a mother may be transmitting, and in another branch a father may be transmitting). In our pedigree series, there are pedigrees with exclusively maternal transmissions, but none with exclusively paternal transmissions. In the Hopkins pedigrees, each family could be categorized as exclusively maternal or exclusively paternal. In this paper we reanalyze our linkage data on chromosome 18, to determine the effect of dividing pedigrees by presence or absence of exclusive maternal transmission, and we observe that it is the non-maternally transmitting pedigrees that show linkage. Table IV of the linkage report by Stine et al. [1995], analyzing affected-sib-pairs, shows the same difference between the pedigree types, overlapping the chromosomal region reported by Berrettini et al. [1991] in their own affected-sib-pair analysis. In Stine et al.

Received for publication August 10, 1995; revision received November 29, 1995.

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[1995] data are also analyzed according to whether the shared alleles are maternal or paternal, but we have not analyzed our data this way for this publication.

METHODS

Pedigree Classification

Pedigree selection and extension, diagnostic procedures and definitions, and affection status models have been described elsewhere [Berrettini et al., 1991]. In addition to the families studied in Bethesda, two families were added, and some additional cases were observed on follow-up, as described elsewhere [Berrettini et al., 1994, 1995].

All families were determined to be unilineal as described [Berrettini et al., 1991]; extension of pedigrees was stopped if a branch became bilineal. The operational definition and procedure were as follows: There were three affection status models described; the major affective disorders are included in models I and II (see below). The third affection status model includes the diagnoses in the first two models and *in addition* includes single episode major depression, cyclothymic personality, hypomania with minor depression, unspecified functional psychosis, hypomania alone, eating disorders, psychiatric hospitalization for other disorder, and schizophrenia. Model III is not used as a definition of who is affected in linkage analyses, but it is used to exclude branches of pedigrees when a person marrying-in has one of these diagnoses. In addition, a branch was excluded if a first-degree relative of the person marrying-in was affected under affection status model I or II (see below for definition). Diagnoses found only in affection status model III were permitted among first-degree relatives of a person marrying in.

For the present analysis, we defined a parent to be transmitting if there was an offspring who was affected, *and* the parent was affected or had a first degree relative (other than offspring) who was affected. This definition was determined to be workable by having persons review the pedigree diagrams in Berrettini et al. [1991], and come to reproducible classification of each transmitting parent. An example of one of these pedigrees, with transmissions designated, is provided in Figure 1. To further help the reader who would like to perform the same classification, the number of observed transmissions for each of these published pedigrees is detailed in Table I. Note that the analysis is of parents who are transmitting, so that no parent is counted more than once even if there were more than one affected offspring.

There are two analyses for which diagnostic classifications were used in this paper: to classify transmitters, and to determine affection status for allele sharing analysis. For both purposes, the previously employed diagnostic hierarchy was used, where BP (or SA) > UP [BP, bipolar; SA, schizoaffective; UP, unipolar (recurrent)].

If a transmitting parent (defined above) or any first degree relative of that parent (except offspring) was BP (or SA), *and* if any offspring was BP (or SA), the transmission was classified as BP to BP. We describe this transmission as BP-BP, and other transmissions as Aff-Aff (includes BP-BP, UP-UP, BP or SA-UP, and UP-BP [or SA]). Suicide in a parent of an affected person was considered a special case in analyzing transmissions: such a parent was considered affected in counting Aff-Aff transmissions. For calculating linkage statistics, there were two affection status models, I and II, corresponding to the affection status models in previous publications [Berrettini et al., 1991]. Model I includes BP, SA, and Model II includes Model I plus UP.

Linkage Analyses

Linkage analysis using the Affected Sib Pair (ASP) method was performed using the SIBPAL program of

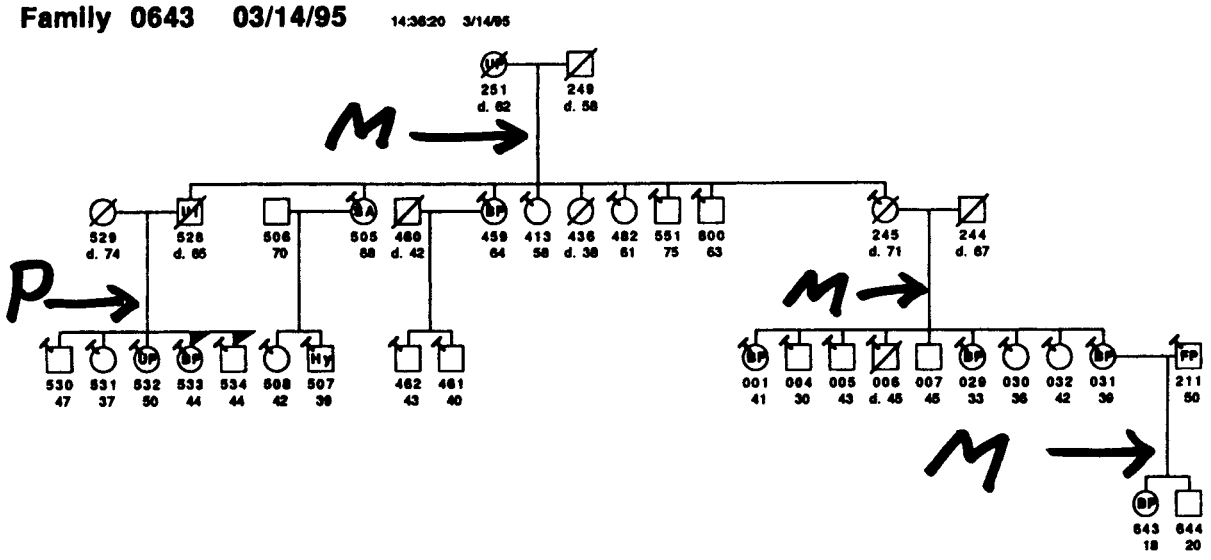

Fig. 1. Example of paternal (P) and maternal (M) transmissions in an extended pedigree. Explanation of method in text.

TABLE I. Maternal and Paternal Transmissions

	Bipolar-bipolar (BP-BP)		Affective-affective (Aff-Aff)	
Family	Paternal	Maternal	Paternal	Maternal
9000	4	1	5	2
9002	0	2	1	2
16	1	1	1	1
48	1	1	2	4
65	0	1	0	1
68	0	2	0	3
92	1	1	2	1
137	0	3	0	3
278	0	3	1	3
441	0	4	0	4
488	0	1	0	1
643	1	2	1	3
1442	1	2	1	2
1482	2	2	2	5
1483	0	1	1	1
1484	0	5	1	5
1505	2	1	2	1
1512	1	1	1	1
1516	3	3	3	4
1520	1	2	1	2
1532	0	2	0	2
1536	1	3	1	3
Sum	19	44	26	54

the S.A.G.E. package [S.A.G.E., 1994] under both affection status models I and II. The Identity by Descent (IBD) score is calculated for all possible pairs of the sibship. The degrees of freedom of the ASP test were corrected to reflect the total number of independent affected pairs.

To determine if the IBD scores for each marker were significantly different in the maternal and paternal pedigrees, a t test comparing sample means, assuming unequal variances was performed [Steele and Torrie, 1980]. Since the IBD statistic is a sample mean of the IBD scores of each sibship, with variance calculated for each sample, this was considered to be the most appropriate test to detect differences in the IBD scores in two independent samples.

The data was also analyzed using the Affected Pedigree Member (APM) method which calculates the Identity By State (IBS) of all the affected members of a pedigree at a particular marker locus [Weeks and Lange, 1988].

RESULTS

Excess Maternal Transmission

Per pedigree analysis. Considering only BP-BP transmitters, there were 10 of 22 pedigrees in which only one sex (mothers only or fathers only) transmitters were observed. One would expect the number of maternal transmission pedigrees to equal the paternal transmission pedigrees, but the observed numbers were 0 and 10 ($\chi^2 = 10$, $P < .01$) (Table I). The results for Aff-Aff show seven pedigrees with only maternal transmitters, and none with only paternal transmitters ($\chi^2 = 7$, $P < .01$).

Per transmitter analysis. Considering the total number of BP-BP transmitters, there were 43 maternal transmitters and 19 paternal transmitters (Table I). This differs from the expected equal numbers ($\chi^2 = 10.08$, 1 df, $P < .002$). For Aff-Aff transmission, the results are similar ($\chi^2 = 9.80$, $P < .002$). The observation of excess maternal transmission initially reported by McMahon et al. [1995] is consistent with these data (see Discussion).

Linkage Analysis on Chromosome 18 According to Pattern of Transmission

McMahon et al. [1995] speculate that imprinting or mitochondrial transmission could account for exclusive maternal transmission in some pedigrees. Although we did not test either hypothesis directly, we did separate out the two types of pedigree in our data, and performed affected-sib-pair linkage analysis on chromosome 18 markers in each set of pedigrees separately. The BP-BP pedigree analysis divided the pedigrees into 10 which were only maternally transmitted (Mat) and 12 which were maternally *and* paternally transmitted (Pat).

18q analysis. In the region of chromosome 18q where Stine et al. [1995] reported possible linkage according to sex of the transmitting parent, the only marker we have studied is D18S64. The Pat pedigrees showed no tendency to linkage under any combination of parental transmission pattern and affection status model. For Mat pedigrees, under affection status model I, BP-BP transmission pattern, the proportion of alleles for the affected sib pairs which were identical by descent (IBD) was 0.63, $P = 0.003$. Under Aff-Aff transmission pattern, there was 0.61 IBD, $P = .008$. For affection status model II, no IBD was significant at this locus.

We also studied another marker not far from this region, D18S42 which did not show any evidence of linkage in any model or transmission pattern.

18p and pericentromeric region analysis. Several markers between D18S62 and D18S56 had previously shown evidence for linkage to affective illness by affected-sib-pairs analysis over all pedigrees. In the subdivided pedigrees, the Mat pedigrees showed little suggestion of linkage, but the *other* (Pat) pedigrees showed increased allele sharing and significant nominal P-values for linkage at multiple loci in this range (Table II). For example, for BP-BP transmission, affection status model II, the Pat pedigree series has nominal P values $\leq .01$ at all loci, but none of the Mat pedigrees do.

Although there is multiple hypothesis testing involved in two types of transmission (BP-BP and Aff-Aff) and two affection status models, the results overall for the Pat and Mat pedigrees are similar in each of the four situations [see Table II and Appendix]. The results of affected-pedigree-member (APM) analyses were similar, with significant results only in the Pat pedigree series (not shown).

A direct test of the significance of the observed increased allele sharing in the Pat pedigrees is to compare the proportion of alleles shared IBD at each locus.

TABLE II. Affected-Sib-Pair Analysis According to Transmission Pattern of Each Pedigree (BP-BP Transmission Used to Assign Pedigree Grouping)*

	Paternal/maternal				Maternal only			
	ASM I		ASM II		ASM I		ASM II	
Marker	IBD	P	IBD	P	IBD	P	IBD	P
D18S62	0.50	NS	0.57	0.01	0.60	0.04	0.58	0.04
D18S21	0.57	0.05	0.59	0.0007	0.53	NS	0.53	NS
D18S37	0.61	0.002	0.57	0.006	0.47	NS	0.51	NS
D18S32	0.66	<0.00001	0.66	<0.00001	0.50	NS	0.51	NS
D18S53	0.64	0.005	0.57	0.01	0.49	NS	0.48	NS
D18S40	0.61	0.03	0.62	0.001	0.52	NS	0.50	NS
D18S45	0.64	0.002	0.60	0.002	0.50	NS	0.52	NS
D18S44	0.56	NS	0.58	0.008	0.48	NS	0.48	NS
D18S66	0.65	0.004	0.60	0.005	0.44	NS	0.47	NS
D18S56	0.67	0.0004	0.63	0.0002	0.50	NS	0.45	NS

* ASM, affection status model. Model I includes BP and SA; model II includes model I and recurrent UP. NS is noted for $P > .05$.

The Pat pedigrees have significantly higher IBD proportions at most loci (Table III). The IBD proportion is consistently higher in the Pat pedigrees, except for the D18S62/D18S21 region. The same pattern is observed for each of the two types of transmission and two affection status models [Table III and Appendix]. For Aff-Aff transmission, three loci show significant differences in shared IBD proportion (D18S32, D18S66, and D18S56).

DISCUSSION

The present results are similar to those of McMahon et al. [1995], who found that in a pedigree series collected for unilineality of apparent illness transmission, there was an excess of transmitting mothers. In an illness with a marked sex difference in prevalence, this might be expected, but in bipolar illness there is little sex difference in the prevalence of affective illness in relatives [Gershon et al., 1982].

We did not attempt to reproduce the analyses of McMahon et al. of morbid risk in all maternal vs. all paternal relatives of the proband, because our series has more distantly extended pedigrees, and not all branches of each pedigree are consistently maternal or paternal.

We cannot assign a genetically precise meaning to the observed pattern of illness transmission. The separation of pedigrees in this manner necessarily must be imprecise, since parents will have different numbers of first-degree relatives. One might attribute the two instances of loci in this study for which maternal pedigrees show $.01 < P < .05$ affected-sib-pair results to such imprecision, although one would not assign any significance to such P values in a regional linkage study, and other possibilities exist.

Bipolar illness does not appear to be a dominant single locus disease, which might be implied in selecting unilineal pedigrees and designating one parent as transmitting and one as non-transmitting. Nor does this set of pedigrees show linkage to chromosome 18 markers under dominant inheritance models we have used, even when subdivided into Mat and Pat pedigrees, although several show positive lod scores (results not shown). In the absence of a genetic model which is consistent with the division of families we use here, the division is a heuristic one.

Nonetheless, it appears that the phenomenon of "excess maternal transmission" has been observed by McMahon et al. [1995], and reproduced here. What could result in the observation? McMahon et al. [1995] mention mitochondrial inheritance and parental imprinting as possibilities. The exclusively maternally transmitted pedigrees do not appear linked to the chro-

TABLE III. Comparison of Allele Sharing Paternal/Maternal vs. Maternal Only Pedigrees*

A. BP-BP transmission: Affection status model I										
Marker (D18 . . .)	S62	S21	S37	S32	S53	S40	S45	S44	S66	S56
Pat. IBD (21)	.50	.57	.61	.66	.64	.61	.64	.56	.65	.67
Mat. IBD (16)	.60	.53	.47	.50	.49	.52	.50	.48	.44	.50
P (Pat = Mat)	NS	NS	0.02	0.009	0.04	NS	0.02	NS	0.03	0.01
B. BP-BP transmission: Affection status model II										
Marker (D18 . . .)	S62	S21	S37	S32	S53	S40	S45	S44	S66	S56
Pat. IBD (42)	.57	.59	.57	.66	.57	.62	.60	.58	.60	.63
Mat. IBD (24)	.58	.53	.51	.51	.48	.50	.52	.48	.47	.45
P (Pat = Mat)	NS	NS	NS	0.004	0.04	0.05	NS	NS	0.04	0.002

* "Pat" refers to pedigrees with paternal and maternal transmission in same pedigree. "Mat" refers to pedigrees with maternal transmission only. Number in parentheses is number of *independent* sib pairs. One sided t-test (Ho: Pat = Mat vs. H1: Pat > Mat) assuming unequal variances [Steel and Torrie, 1980].

mosome 18 markers we studied, and mitochondrial inheritance or maternal imprinting of illness susceptibility genes remains possible. Additional possibilities for excess maternal inheritance include artifacts of the diagnostic process, such as differential disclosure of illness in offspring by males and females who are parents. This explanation includes non-disclosure to their relatives as well as to the researchers. Yet another possibility is differential gene-environment interactions according to whether the transmitting parent is a male or a female, such as an intrauterine environmental event, or possible childhood developmental effects dependent on maternal illness.

The major difference between the family transmission patterns of illness in this report and in McMahon et al. [1995] is that they observe pedigrees with paternal transmission only, and we do not observe kindreds in which transmission is limited to the paternal type. This appears to be related to the further extension of pedigrees in this series. As is evident in Table I, the larger pedigrees tend to have more maternal/paternal (Pat) transmission. If there is a bias for non-maternal pedigrees to be larger (with more affected individuals), they would tend to be more informative for linkage. But this bias should not have produced significantly different IBD scores.

A provocative interpretation of the present linkage analyses is that two different genetic transmission patterns are separated to a considerable extent by the division of pedigrees into Mat and Pat (maternal and maternal/paternal), and that it is the Pat pedigrees which show linkage by non-parametric analyses in the chromosome 18 pericentromeric region. This leaves open the question of transmission in the maternal inheritance pedigrees, which by one definition (BP-BP transmission) represent almost half the pedigrees in our series.

It is perhaps discountable that significant linkage results on chromosome 18 were not observed in the Mat pedigrees, because the sample size is small. The key comparison supporting different chromosome 18 related inheritance, between Mat and Pat pedigrees, is the difference in allele sharing. The small sample size in one group would tend to obscure a significant difference, but differences are observed nonetheless.

Are the present results consistent with the report of Stine et al. [1995]? Only affected-sib-pair analysis according to transmission pattern is presented in both papers. In Table 4 of Stine et al., the pedigrees are divided according to paternal or maternal transmission, and then separate columns are provided for IBD for each parent's allele source. In the analysis of Stine et al. of shared alleles from *either* parent, in Table 4, the results are very similar to our own. The maternal pedigrees do not show significant allele sharing by any measure, but the paternal pedigrees show sharing over a large region, with the smallest P value (.0002) at D18S37. The comparable P value at the same locus in these data is .006, and the nearby locus D18S32 has P value of $<.00001$ (Table II and Fig. 2, in this paper).

Other analyses in Stine et al. [1995] suggest a locus on 18q. At this point, one can conclude that linkage on chromosome 18 and the pattern of transmission are strongly implied by the two papers, and that the precise location of the locus or loci remains to be determined.

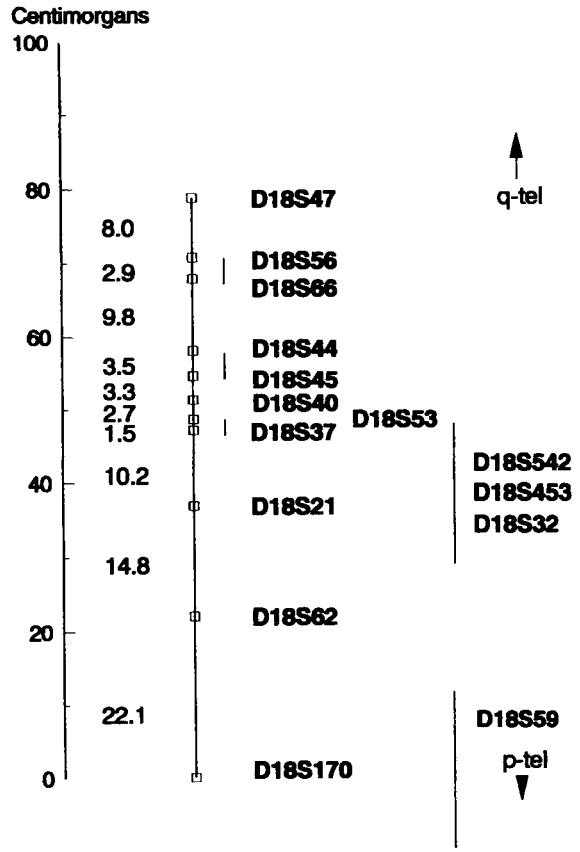

Fig. 2. Map of pericentromeric markers of chromosome 18 in this pedigree series, constructed with the CRI-MAP program.

If this finding continues to prove reproducible, clinical data in BP illness could be "purified" by subdividing according to the distribution of diagnoses in the families. The proportion of alleles IBD in the linkage region is thereby much increased. As shown by Kruglyak and Lander [1995], as the sharing proportion of alleles in affected relatives pairs increases, the number of such pairs needed to resolve the linkage region to a 1 cm interval became smaller. Genetic subdivision of an illness by clinical or pedigree configuration criteria can thus play an important role in discovery of disease susceptibility mutations.

ACKNOWLEDGMENTS

The advice and computations of Dr. Lynn Goldin and the advice of Dr. Alan Sanders are gratefully acknowledged. This work was supported in part by a NARSAD grant to WHB and by NIMH grant 49181 to WHB. Family 9002 was included with permission from Indiana University investigators Dr. John I. Nurnberger, Jr., and Marvin Miller. Some of the results of this paper were obtained by using the program package S.A.G.E., which is supported by a U.S. Public Health Service Resource Grant (1 P41 RR03655) from the Division of Research Resources.

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Appendix A. Affected-Sib-Pair Analysis According to Transmission Pattern of Each Pedigree (Aff-Aff Transmission Used to Assign Pedigree Grouping)

	Paternal and maternal				Maternal only			
	ASM I		ASM II		ASM I		ASM II	
Marker	IBD	P	IBD	P	IBD	P	IBD	P
D18S62	0.51	NS	0.57	0.009	0.61	NS	0.58	NS
D18S21	0.53	NS	0.57	0.007	0.62	NS	0.60	NS
D18S37	0.60	0.005	0.56	0.009	0.48	NS	0.52	NS
D18S32	0.65	<0.00001	0.66	<0.00001	0.50	NS	0.51	NS
D18S53	0.62	0.01	0.56	0.03	0.50	NS	0.50	NS
D18S40	0.60	0.03	0.60	0.003	0.51	NS	0.51	NS
D18S45	0.62	0.003	0.59	0.004	0.50	NS	0.54	NS
D18S44	0.53	NS	0.56	0.03	0.53	NS	0.53	NS
D18S66	0.64	0.006	0.60	0.005	0.44	NS	0.45	NS
D18S56	0.68	0.00009	0.61	0.0003	0.44	NS	0.42	NS

Appendix B. Comparison of Allele Sharing in Paternal/Maternal vs. Maternal Only Pedigrees

A. Aff-Aff transmission: Affection status model I										
Marker (D18S . . .)	62	21	37	32	53	40	45	44	66	56
Pat. IBD (25)	.51	.53	.60	.65	.62	.60	.62	.53	.64	.68
Mat. IBD (12)	.61	.62	.48	.50	.50	.51	.50	.53	.44	.44
P (Pat 5 Mat)	NS	NS	NS	.02	NS	NS	NS	NS	.03	.005
B. Aff-Aff transmission: Affection status model II										
Marker (D18S . . .)	62	21	37	32	53	40	45	44	66	56
Pat. IBD (47)	.57	.57	.57	.66	.56	.60	.59	.56	.60	.62
Mat. IBD (19)	.58	.60	.53	.50	.50	.51	.54	.53	.45	.42
P (Pat 5 Mat)	NS	NS	NS	0.008	NS	NS	NS	NS	0.03	0.0008